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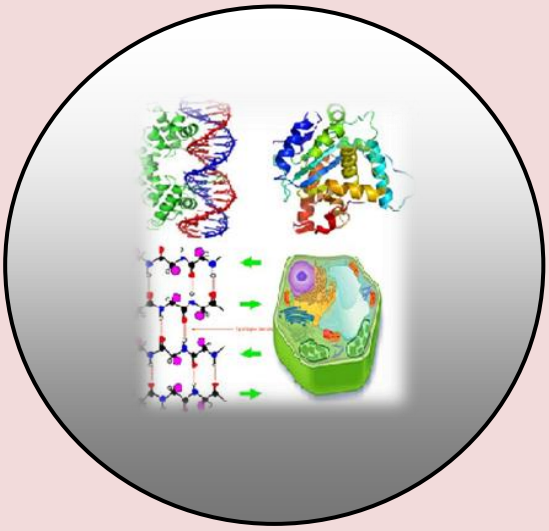
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 37 (1) 2020 Pages No. 84-90



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 37, No. 1: 84-90, 2020
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 37/01/1020/2020

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

http:// www.sasjournals.com
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RESEARCH PAPER
Received: 10/12/2019 Revised: 01/03/2020 Accepted: 02/03/2020

Effectiveness of *Mansoa alliacea* Leaf Extract as Botanical Pesticides to Control *Colletotricum acutatum* Agent Anthracnose Disease on Chili Pepper

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ABSTRACT

Research on Effectiveness of Mansoa alliacea Leaf Extract as Botanical Pesticides to Control Colletotrichum acutatum Agent Anthracnose Disease on Chili Pepper has been conducted. The results showed that the Mansoa alliacea leaf extract treatment significantly (P <0.05) decreased the percentage of anthracnose disease in chili pepper. Treatment of extract with concentration between 1% to 5% able to inhibitory of anthracnose disease percentage between 30,39% until 100%, while the use of extract 4% and 5% no significant difference (P> 0,05) to the percentage disease. There was negative correlation between extract concentration as anthracnose disease percentage, where the higher concentration of extract formula, the lower the percentage of disease with the equation y = - 25,86ln (x) + 42,69 with coefficient of determination (R2) = 0,97. Treatment of Mansoa alliacea leaf extract significantly (P <0.05) decreased the intensity of anthracnose disease in chili pepper. Treatment of extract with concentration between 1% to 5% can decrease disease intensity between 34,31% until 100%, while the use of extract 4% and 5% no significant different response (P> 0,05) to disease intensity. This study proves that there is a negative correlation between extract concentration as anthracnose disease intensity in chili pepper with the equation y = - 25,07ln (x) + 39,16 with coefficient of determination (R2) = 0,99. The higher of concentration of extract used, the result will increase until the optimum concentration (4%), and if the concentration of Manosa alliacea leaf extract used is higher than 4% concentration then the productivity of chili pepper will decrease according to the equation y = -0,013x2 + 0,199x + 0.020 with the coefficient of determination (R2) = 0.85.

Keywords: Effectiveness, Anthracnose, Disease intensity and Mansoa alliacea.

INTRODUCTION

Colletotrichum acutatum is the fungus that most often infects and causes symptoms of anthracnose disease in chili pepper (Sudiarta and Sumiartha, 2012; Sudirga, 2015). According to Semangun (2007) anthracnose disease can cause a decrease yield in chili pepper to 50%. Synthetic fungicides are still often used to control anthracnose diseases, if their use continuously it can cause side effects such as environmental pollution, pathogen resistance and accumulation of toxic compounds in agricultural products.

According to Sa'id (1994) pollution of pesticides is harmful to humans, animals and the environment as well as agricultural products. Based on these problems, anthracnose disease control needs to be sought alternatives by using botanical fungicides which are not harmful to consumers or the environment. According to Sudirga et al. (2018) leaf extract of *Mansoa alliacea* can inhibit the growth of *Colletotrichum acutatum* that cause anthracnose disease in chili pepper.

Based on these problems, a study was carried out on "effectiveness of *Mansoa alliacea* leaf extract as botanical pesticides to control *Colletotricum acutatum* agent anthracnose disease on chili pepper ". The results of this study are expected to find effective plant pesticides to control anthracnose diseases in chili pepper, so as to reduce the use of synthetic pesticides.

MATERIALS AND METHODS

Place and time of research

This research was conducted at the Biopesticide Laboratory of the Faculty of Agriculture of Udayana University (in vitro test) and in the Experimental Garden Greenhouse of the Faculty of Agriculture of Udayana University (in vivo test). Preliminary research was carried out in March to July 2018 and further research was carried out from September 2019.

Isolation of *Colletotrichum acutatum*

Colletotrichum acutatum isolated from chili pepper that show symptoms of anthracnose disease from cultivated chili. Chili pepper that show symptoms of anthracnose disease are cleaned with running water followed by sterile water and then cut to size 1 cm x 1 cm then placed on PDA media. The growing fungus is then isolated and purified and identified, and to ensure that the isolated and purified fungus causes anthracnose disease in chili pepper, the Koch Postulate test is carried out. The fungus isolates were then maintained on a sloping PDA media and ready to be used for further reserach.

Extraction of leaf *Mansoa alliacea*

Mansoa alliacea leaves used were obtained around garden of Sanglah Hospital Denpasar. Extraction is done by chopping the leaves of *Mansoa alliacea* into smaller parts, then dried at room temperature, and after drying, this material is made into powder by blending. *Mansoa alliacea* leaf powder (100 grams) was then macerated with 1,000 ml of methanol PA (Pro Analysis) for 72 hours. The filtrate was obtained by filtering and then evaporated using a vaccum rotary evaporator (Iwaki, Japan) at 40oC, to obtain crude extract used for further research.

Greenhouse experiment

Greenhouse research was carried out using a Randomized Block Design (RBD) consisting of 6 treatments namely: F0 = Control (without extract), F1 = *Mansoa alliacea* leaf extract 1%, F2 = *Mansoa alliacea* leaf extract 2%, F3 = *Mansoa alliacea* leaf extract 3%, F4 = *Mansoa alliacea* leaf extract 4%, F5 = *Mansoa alliacea* leaf extract 5%, each treatment was repeated 4 times and each treatment unit contained 6 polybags /plant. The research included: seed sowing, preparation of planting media, seedlings planting, fertilizing, plant maintenance, inoculation of pathogenic fungi (*Colletotrichum acutatum*.), Application of *Mansoa alliacea* leaf extract formula, observing the effect of extracts on anthracnose and harvest symptoms. The parameters observed included the percentage of anthracnose disease, the intensity of anthracnose disease and the yield of chili pepper.

RESULTS AND DISCUSSION

RESULT

The effectiveness of *Mansoa alliacea* leaf extract against anthracnose disease in chili pepper in vivo was carried out in a greenhouse. The concentration of *Mansoa alliacea* leaf extract tested was 5%, 4%, 3%, 2%, 1% and 0% as a control. The results showed that the treatment of *Mansoa alliacea* leaf extract significantly ($P < 0.05$) decreased the percentage of anthracnose disease in chili pepper as presented in Table & Figure 1.

Table 1. Effect of treatment of *Mansoa alliacea* leaf extract on percentage of anthracnose disease in chili pepper.

Konsentrasi ekstrak (%)	Persentase penyakit (%)	Daya hambat dibandingkan dengan kontrol (%)
0	58,90[a*]	-
1	41,00[b]	30,39
2	26,07[c]	55,74
3	18,43[d]	68,71
4	4,13[e]	92,99
5	0[e]	100

The average value followed by the same letter indicates no different based on Duncan's Multiple Range Test at 5% level

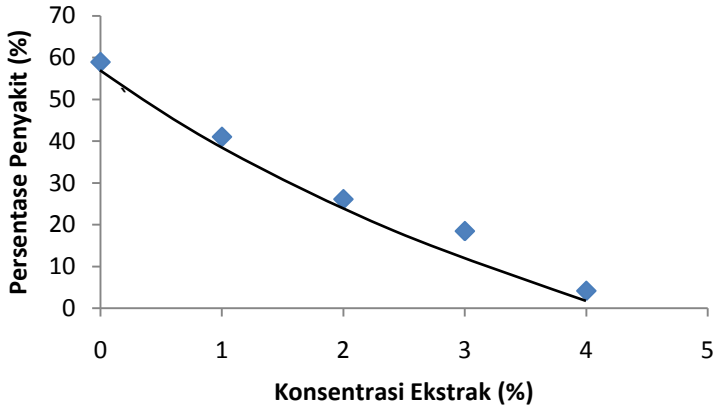

Figure 1. The graph of the relationship between the concentration of *Mansoa alliacea* leaf extract and the percentage of anthracnose disease in chili pepper.

The treatment of *Mansoa alliacea* leaf extract significantly (P <0.05) reduced the intensity of anthracnose disease in chili pepper, extract treatment with concentrations between 1% to 5% was able to reduce disease intensity between 34.31% to 100%, while the use of extract 4% and 5% gave a response that was not significantly different (P> 0.05) to the intensity of the disease as presented in Table & Figure 2.

Table 2. Effectiveness of Mansoa alliacea leaf extract on intensity anthracnose disease in chili pepper.

Konsentrasi ekstrak (%)	Intensitas penyakit (%)	Daya hambat dibandingkan dengan kontrol (%)
0	58,17[a*]	-
1	38,21[b]	34,31
2	24,58[c]	57,74
3	10,41[d]	82,10
4	2,59[e]	95,55
5	0[e]	100

The average value followed by the same letter indicates no different significantly based on Duncan's Multiple Range Test at 5% level.

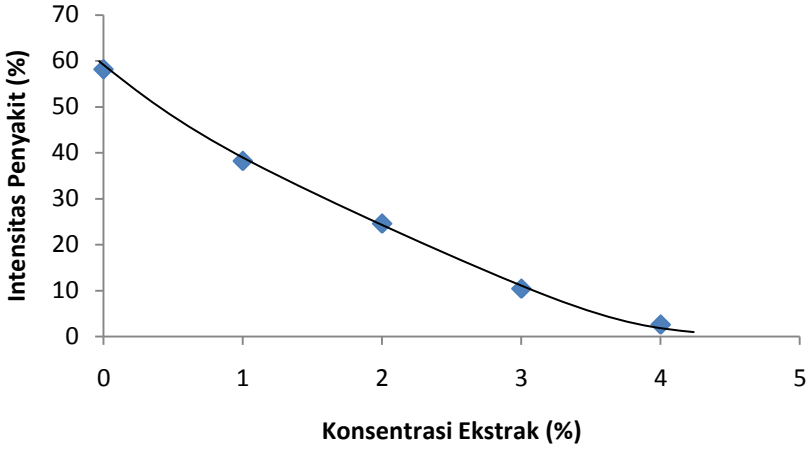

Figure 2. The graph of the relationship between the concentrations of *Mansoa alliacea* leaf extract with the intensity of anthracnose disease in chili pepper.

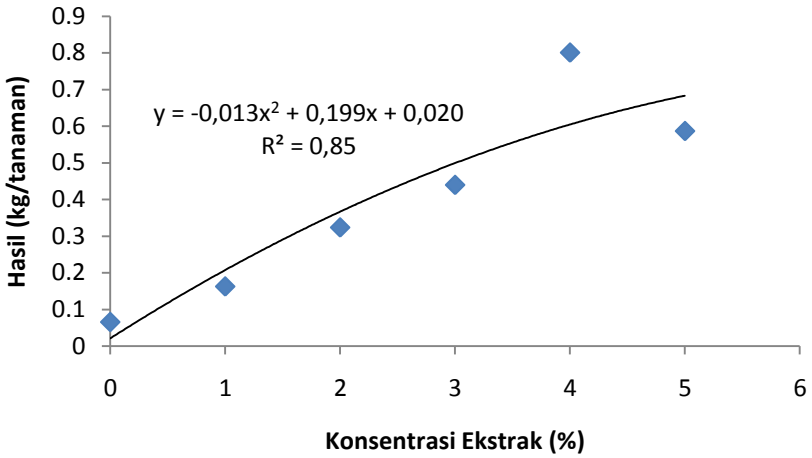

Figure 3. Graph of the relationship between the treatment of leaf extract concentration *Mansoa alliacea* with the results of planting chilies.

Table 3. The results of chili pepper with *Mansoa alliacea* leaf extract treatment.

Konsentrasi ekstrak (%)	Hasil (kg/tanaman)	Kehilangan hasil yang dapat diselamatkan dibandingkan dengan kontrol (%)
0	0,066^{a*}	-
1	0,163^{a}	59,51
2	0,324^{b}	79,63
3	0,440^{c}	85,00
4	0,801^{e}	91,76
5	0,587^{d}	88,76

The average value followed by the same letter indicates no different based on Duncan's Multiple Range Test at 5% level

The results of the study in the greenhouse in Table and Figure 3, show that the higher the concentration of *Mansoa alliacea* leaf extract used, the chili pepper yield and the percentage of yield loss that can be saved increases. The treatment of extract 1% was not statistically significantly different (P> 0.05) with control, and the treatment of extract 5% with the intensity of the disease 0% was not statistically significant (P> 0.05) with the treatment of extract 4% with the intensity of disease 2,59%, but the yield of chili pepper at 4% extract treatment was 0.801 kg / plant significantly different (P <0.05) with 5% extract treatment with a yield of 0.587 kg / plant.

DISCUSSION

Based on the results of the studies in Tables 1, 2, 3 and Figure 1, 2, 3 shows that, the treatment of *Mansoa alliacea* leaf extract with a concentration of 4% (w / v) is effective in reducing the intensity of anthracnose disease in chili pepper and reducing yield losses by 91.76 % with yield of 0.801 kg / plant. If the yield is converted in tons / ha with a total number of 25,000 plants per hectare of chili, the productivity of chili pepper treated with *Mansoa alliacea* leaf extract with a concentration of 4% reaches 20.02 tons / ha. These results are almost the same as reported by Syukur et al. (2012) that the productivity potential of large superior chili varieties in Indonesia reaches 20 tons / ha.

According to Suprapta (2014) the use of plant extracts in high concentrations has the potential to cause plant poisoning (phytotoxic). This is because in plant extracts in addition to active compounds there are also other compounds that are polar or non-polar. Compounds that are non-polar if there is an excessive amount will cause the dissolution of the waxy layer in plants, the waxy layer acts as a plant protector. Hossain et al. (2012) reported that the weight of pea seeds produced per plant in the treatment of *Moringa oleífera* root extract concentration was 5% higher when compared to the 10% extract treatment. A similar study was reported by Nashwa and Elyours (2012) that the treatment of 1% concentration of *Nerium oleander* leaf extract produced more tomatoes than the 5% concentration of *N. oleander l*eaf extract treatment.

The control of anthracnose disease in cultivated plants caused by the fungus *Colletotrichum acutatum* by using a plant-based fungicide has been carried out. Gawade at al. (2014) reported that *Aegle marmelos* (L) leaf extract can inhibit the growth of the fungus *C. acutatum* with a zone of inhibition zone of 22 mm. Nogodula et al. (2012) reported that, crude extracts of *Mansoa alliacea* leaves were able to inhibit the growth of *Canida albican* fungi with inhibition zone diameters of 16.67 mm. Whereas Sudirga et al. (2014) reported that the crude extract of *awar-awar* leaves was effective in inhibiting the growth of *C. acutatum* mushroom with inhibition zone diameter of 30 mm. Awar-awar leaf extract significantly inhibits colony growth, spore density, spore germination, and *C. acutatum* fungal cell biomass. This is caused by the ability of awar-awar leaf extract to inhibit the growth of hyphae and spores of *C. acutatum* by damaging the permeability of cell membranes so that the hypha cells and fungal *C. acutatum* spores undergo lysis or crenation.

Based on the results of phytochemical tests on the active fraction of positive *Mansoa alliacea* leaf extracts containing phenolic compounds, alkaloids and terpenoids. The mechanism of phenolic compounds is by forming a complex of bonds to membrane proteins, modifying membrane proteins, or forming a protein-phenol complex which involves the presence of hydrogen bonds between proteins and phenols. This bonding complex can cause damage (denaturation) of hydrogen bonds in the fungal cell wall, so that it can interfere with membrane permebility and disrupt cell metabolism by inhibiting the transport of nutrients in and out of cells. Besides that phenolic compounds can denaturate protein bonds in the cell membrane so that the cell membrane becomes lysis.

Phenolic compounds can inhibit the formation of the enzyme C-demethylase which plays a role in the synthesis of ergosterol and inhibits chitin synthesis in fungal cell walls. So that it can cause permeability disruption in the form of potassium ion leakage and damage to cell wall permeability that causes the cell contents to come out or the intracellular matrix of the fungus to come out, resulting in cell leakage that causes fungal cell chemistry (Hada and Sharma, 2014). While the mechanism of action of alkaloids and terpenoids compounds contained in the extracts of Mansoa alliacea leaf is an alkaloid group of compounds working by inhibiting the synthesis of nucleic acids and affecting the fungal cell wall.

Alkaloids are compounds that have antimicrobial activity, namely by inhibiting the enzyme DNA and RNA polymerase, damaging cell membranes and denaturing proteins. Whereas terpenoid compounds have antifungal activity by influencing cell membrane permeability that can cause lysis of fungal cells, besides that it can inhibit the synthesis of ergosterol which is a component of fungal cell walls so that fungi die due to incompletely formed cell walls (Diana et al., 2014; Karo et al., 2017).

CONCLUSION

Mansoa alliacea leaf extract at a concentration of 4% (w / v) was effective in suppressing anthracnose disease in chili pepper plants with a suppression of disease percentage of 92.99%, disease intensity of 95.55% and yield loss that could be saved was 91.76% if compared to control.

ACKNOWLEDGEMENTS

My thanks go to the Director General of Higher Education and Chancellor of Udayana University for the assistance of research funds through the PDUPT (Higher Education Basic Research) Fund and the Head of the Biology Laboratory of the Program Study of Biology, Faculty of Natural Sciences, Udayana University for the facilities provided during the research.

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